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<p>(21) International Application Number: PCT/GB92/01291 (22) International Filing Date: 15 July 1992 (15.07.92) (30) Priority data: 9115245.4 16 July 1991 (16.07.91) GB (71) Applicant (for all designated States except US): IMPERIAL CHEMICAL INDUSTRIES PLC [GB/GB]; Imperial Chemical House, Millbank, London SW1P 3JF (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): LIEBERGESELL, Matthias [DE/DE]; Institut für Mikrobiologie, Georg-August-Universität, Grisebachstraße 8, D-3400 Göttingen (DE). STEINBÜCHEL, Alexander [DE/DE]; Institut für Mikrobiologie, Georg-August-Universität, Grisebachstraße 8, D-3400 Göttingen (DE).</p>		<p>(74) Agent: HUSKISSON, Frank, Mackie; Imperial Chemical Industries plc, ICI Group Patent Department, P.O. Box 6, Bessemer Road, Welwyn Garden City, Herts AL7 1HD (GB). (81) Designated States: AU, BB, BG, BR, CA, CS, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). Published With international search report.</p>
<p>(54) Title: PRODUCTION OF POLYALKANOATE</p> <p>(57) Abstract</p> <p>Genes encoding polyhydroxyalkanoate synthase, β-ketothiolase and acetoacetyl CoA reductase are isolated from the publicly available bacterium <i>Chromatium vinosum</i>. Recombinant genomes of plants or other species of bacteria which contain these genes are capable of producing polyalkanoate polymers. The nucleotide sequences of the said three genes have been determined.</p>		

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PRODUCTION OF POLYALKANOATE

This invention relates to the production of polyhydroxyalkanoate by the culture of microorganisms which produce same.

Poly-3-hydroxybutyrate is a linear polyester of D(-)-3-hydroxybutyrate. It was first discovered in Bacillus megaterium in 1925. Polyhydroxybutyrate accumulates in intracellular granules of a wide variety of bacteria. The granules appear to be membrane bound and can be stained with Sudan Black dye. The polymer is produced under conditions of nutrient limitation and acts as a reserve of carbon and energy. The molecular weight of the polyhydroxybutyrate varies from around 50,000 to greater than 1,000,000, depending on the microorganisms involved, the conditions of growth, and the method employed for extraction of the polyhydroxybutyrate. Polyhydroxybutyrate is an ideal carbon reserve as it exists in the cell in a highly reduced state, (it is virtually insoluble), and exerts negligible osmotic pressure.

Polyhydroxybutyrate and related poly-hydroxyalkanoates, such as poly-3-hydroxyvalerate and poly-3-hydroxyoctanoate, are biodegradable thermoplastics of considerable commercial importance.

The term "polyhydroxyalkanoate" as used hereinafter includes copolymers of polyhydroxybutyrate with other polyhydroxyalkanoates such as poly-3-hydroxyvalerate.

Polyhydroxyalkanoate is biodegradable and is broken down rapidly by soil microorganisms. It is thermoplastic (it melts at 180°C) and can readily be moulded into diverse forms using technology well-established for the other thermoplastics materials such as high-density polyethylene which melts at around the same temperature (190°C). The material is ideal for the production of biodegradable packaging which will degrade in landfill sites and sewage farms. The polymer is biocompatible, as well as biodegradable, and is well tolerated by the mammalian, including human, body, its degradation product, 3-hydroxybutyrate, is a normal mammalian metabolite. However, polyhydroxyalkanoate degrades only slowly in the body and its medical uses are limited to those applications where long term degradation is required.

Polyhydroxyalkanoate, produced by the microorganism Alcaligenes eutrophus, is manufactured, as a copolymer with of polyhydroxybutyrate and polyhydroxyvalerate, by Imperial Chemical Industries PLC and sold under the Trade Mark BIOPOL. It is normally supplied in the form of pellets for thermoprocessing. However, polyhydroxyalkanoate is more expensive to manufacture by existing methods than, say, polyethylene. It is, therefore, desirable that new, more economic production of polyhydroxyalkanoate be provided.

An object of the present invention is to provide materials and a method for the efficient production of polyhydroxyalkanoate.

According to the present invention there is provided gene fragments isolated from the bacterium Chromatium vinosum and encoding PHA polymerase, acetoacetyl CoA reductase and β -ketothiolase.

5 Preferably the C.vinosum is of the strain designated D, available to the public from the Deutsche Sammlung für Mikroorganismen under the Accession Number 180.

10 The invention also provides a 16.5kb EcoRI fragment of C.vinosum DNA, designated PP10, hybridizable to a 5.2kb SmaI/EcoRI fragment, designated SE52 isolated from Alcaligenes eutrophus and known to contain all three of said genes responsible for expression of PHAs.

15 The invention further provides a fragment of the said PP10 fragment, designated SE45, encoding the PHA-synthase and β -ketothiolase genes and a region, designated SB24, encoding the acetoacetyl CoA reductase gene.

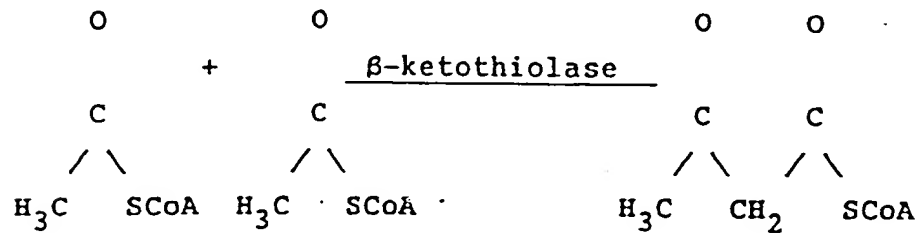
20 The invention also provides a recombinant genome of a microorganism, preferably a bacterium or a plant, which contains one or more of said fragments designated PP10, SE45 and region SB24.

25 Finally, the invention provides a method for the manufacture of PHAs, comprising culturing the microorganism Chromatium vinosum, or a bacterium of a different species having stably incorporated within its genome by transformation one or more PHA synthesising genes from Chromatium vinosum.

30 The biosynthesis of polyhydroxyalkanoate from the substrate, acetyl-CoA involves three enzyme-catalysed steps.

The three enzymes involved are β -ketothiolase, acetoactyl-CoA-reductase and polyhydroxy-

butyrate-synthase, the genes for which have been cloned from Chromatium vinosum. The three genes are known to facilitate production of polyhydroxyalkanoates, the reactions involved being represented as follows:.

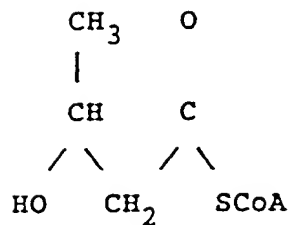


NADPH

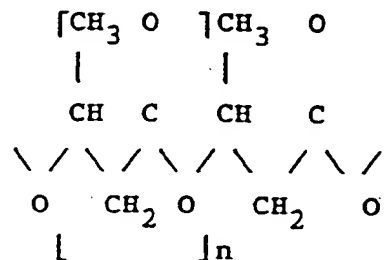
NADP-linked

Acetoacetyl-CoA Reductase

NADP



Polyhydroxybutyrate-
synthase



The invention will now be described with reference to the accompanying drawings, of which;

Figure 1 is the physical map of the 16.5 kb EcoRI fragment of Chromatium vinosum DNA, designated PP10. The positions of the restriction sites and positions and names of the sub-fragments are shown. PHA-synthase and β -ketothiolase genes are located in fragment SE45 and acetoacetyl CoA reductase in region SB24;

Figure 2 is the map of PP10 showing the positions of the β -ketothiolase and acetoacetyl CoA reductase genes and of the PHA-synthase gene open reading frames ORF2 and ORF3.

Figure 3 is the complete nucleotide sequence of fragment SE45. The transcriptional start sites and terminators for the β -ketothiolase gene and for ORF3 and ORF 3 are shown. The positions of the "-10" and "-35" sequences are also shown, as are the positions of the putative ribosome binding sites ("s/d"). Translational start and stop (*) codon are also marked and the amino acid sequences of the β -ketothiolase, ORF2 and ORF3 are given.

Figure 4 shows the alignment of the amino acid sequences of Chromatium vinosum ORF3 with PHA polymerase of Alcaligenes eutrophus and PHA polymerases 1 and 2 of Pseudomonas oleovorans.

Figure 5 shows the complete nucleotide sequence of the DNA encoding PHA synthesis genes from Chromatium vinosum. The positions of PHA polymerase (phbC), acetoacetyl CoA reductase (phbB) and ketothiolase (phbA) genes are shown, and also the positions of ORF2, ORF4, ORF5 and ORF7.

Figure 6 shows the alignment of the amino acid sequences of ketothiolases encoded by C.vinsoum

(C.v.), A.eutrophus (A.e.), Zoogloea ramigera (Z.r.), Escherichia coli (E.c.), Saccharomyces uvarum (S.u.) and Rattus norvegicus (R.n.)

5 Figure 7 shows the alignment of the amino acid sequences of acetoacetyl CoA reductases encoded by C.vinosum (C.v.), A.eutrophus (A.e.) and Z.ramigera (Z.r.)

10 Figure 8 is a Table (Table 1) showing the heterologous expression in Escherichia coli of DNA fragments from C.vinosum. Activities of PHA biosynthetic enzymes expressed by the different fragments are shown. The levels of PHA accumulated in E.coli transformed with the fragments are also given.

15 EXAMPLE

The organism C.vinosum was a gift from Dr J. Imhoff of the University of Bonn, Germany.

1. Isolation of DNA fragments from C.vinosum encoding PHA synthesis genes

20 A 5.2 kb SmaI/EcoRI fragment (SE52), which codes for all three PHA biosynthetic genes has previously been isolated from Alcaligenes eutrophus [Schubert et al., J. Bacteriol. 170 (1988)]. This fragment was used to detect PHA biosynthetic genes of C.vinosum. EcoRI restricted genomic DNA of C.vinosum was blotted on to a nylon membrane and hybridized with biotinylated SE52 DNA. One signal appeared, representing a DNA fragment of 16.5 kb.

25 A λ L47 gene bank from C.vinosum genomic DNA was prepared and plates with approximately 800 plaques were blotted on to nylon membranes and hybridized with biotinylated SE52 DNA. One positive recombinant phage was isolated, which harboured a 16.5 kb EcoRI fragment, which was

designated PP10 (Figure 1). With PP10 and a 9.4 kb EcoRI/PstI subfragment (EP94) of PP10, the phenotype of the wild type could be restored in PHA-negative mutants of A.eutrophus.

5 Expression studies in E.coli (see below) showed that a 4.5 kb SmaI/EcoRI (SE45) subfragment of EP94 encodes for PHA synthase and β -ketothiolase. The nucleotide sequence of this fragment was determined by the dideoxy-chain
10 termination method of Sanger et al. with alkaline denatured double stranded plasmid DNA. The T7-polymerase sequencing kit of Pharmacia, Uppsala, Sweden, was used with 7-deazaguanosine-5'-tri-phosphate instead of dGTP. Most of the sequence
15 was determined with a set of unidirectional overlapping deletion clones generated by exonuclease III digestion. For sequencing regions which were not covered by the deletion plasmids synthetic oligonucleotides were used.

20 It was not possible to clone the 4.9 kb SmaI/PstI fragment PS49 in a multi-copy vector. Therefore, fragment EP94 (Figure 1) was treated with Exonuclease Bal31, ligated to Bluescript SK and transferred to E.coli Xl-1 Blue. A clone was
25 isolated which harboured Bluescript SK with a 5.5 kb fragment (B55) and which expressed β -ketothiolase and NADH-dependent reductase activity. 3146 base pairs of B55 were part of the SE45 fragment. The other part (approximately 2350
30 base pairs, SB24) has been sequenced applying the primer hopping strategy. The sequence and the position of the reductase gene on SB24 are known. The results of these studies, including the organisation of the PHA biosynthetic genes in

C.vinosum and the sites of the ketothiolase, reductase and PHA synthase genes are shown in Figure 2. The determination of the full sequence of SB24 is in progress.

5 2. Sequence analysis of the C.vinosum PHB Synthetic Genes

 The nucleotide sequence of SE45 is shown in Figure 3. The fragment size of SE45 is 4506 bp.

2.1 PHB synthase

10 The fragment sequence corresponding to the PHB synthase gene is designated as ORF3. The determination of synthase activity of deleted plasmids containing SE45 (See below) gave evidence that expression of ORF2 is also required for
15 expression of synthase activity.

 ORF2 and ORF3 are transcribed as an operon. The determination of the transcription start site of ORF2 was conducted by S1 nuclease mapping. This site occurs at bp 3059 from the 3' end of SE45. A
20 putative "-10" site, given as 5'-ACAGAT-3' occurs at bp 3073-3078, and a "-35" site occurs at bp 3092-3099. A putative ribosome binding site occurs at bp 3040-3045. The translation start codon commences at bp 3030. The translation stop codon
25 occurs at bp 1958.

 The putative ribosome binding site of ORF3 occurs at bp 1907-1911. The translation start ATG for ORF3 occurs at bp 1899, and the translation stop codon at bp 833. Putative transcriptional
30 terminator sites occur at hairpin structures at bp 773-786 and 796-823.

 ORF2 encodes a polypeptide of 358 amino acids with a MW of 40525 da. ORF3 encodes a polypeptide of 356 amino acids with a MW of 39739 da. The gene

size of ORF3 is approximately 30% smaller as compared with the PHA polymerase genes of A.eutrophus and P. oleovorans. The alignments of the primary structures of C.vinosum PHA polymerase, A.eutrophus PHA polymerase and P.oleovorans PHA polymerases 1 and 2 are shown in Figure 4. Thus the ORF3 C.vinosum polymerase is shorter than the other polymerase enzymes, lacking the first 172 amino acids from the NH₂ terminus of the A.eutrophus PHA polymerase, and the first 148 amino acids of the Pseudomonas polymerases. The amino acid sequence of ORF3 exhibited an overall homology of 25% to the polymerase of A.eutrophus, with certain discrete regions of conserved sequence.

The amino acid sequence of ORF2 showed no significant homology to other enzymes in the NBRF gene bank.

2.2 β ketothiolase

The β ketothiolase and acetoacetyl CoA reductase genes are transcribed in opposite direction to ORF2 and ORF3 (Figure 2). A "-10" site in the identified ketothiolase promoter occurs at bp 3105-3111, and a "-35" site at bp 3082-3086. A putative ribosome binding site occurs at bp 3167-3171. The translation starts signal occurs at bp 3181. The translation stop codon occurs at bp 4361.

The alignments of the primary structures of β ketothiolases from Chromatium vinosum and other sources are shown in Figure 5. Considerable homology is apparent between the amino acid sequences of ketothiolases from C.vinosum and other bacterial and non-bacterial sources.

2.3 Acetoacetyl CoA reductase

The alignments of the primary structures of acetoacetyl CoA reductases from C.vinosum, A.eutrophus and Z.ramigera are shown in Figure 6.
5 All three reductases are of similar chain length, while considerable homology is apparent between the sequences of reductases from these bacteria.

The Chromatium vinosum PHA synthetic genes therefore differ from the PHA synthetic genes of A.eutrophus and P.oleovorans in the following
10 respects:

i) Whereas A.eutrophus PHB polymerase, acetoacetyl CoA reductase and β ketothiolase genes are all transcribed as an operon, in C.vinosum the
15 ketothiolase and reductase genes are transcribed separately from the polymerase, and are transcribed in the opposite direction to the polymerase ORF3 and ORF2 genes.

ii) In contrast to A.eutrophus, where one
20 gene product is required for polymerase activity, in C.vinosum two gene products, represented by ORF2 and ORF3 are required for expression of polymerase activity.

iii) The C.vinosum ORF3 polymerase is 172
25 amino acids shorter, at the amino terminus, than the A.eutrophus polymerase, and 148 amino acids shorter than the P.oleovorans polymerases 1 and 2. The C.vinosum ORF3 shows only 25% homology with the primary sequence of the A.eutrophus polymerase.

iv) The A.eutrophus acetoacetyl CoA reductase
30 enzyme involved in PHB synthesis is NADPH specific, while the C.vinosum enzyme exhibits a marked preference for NADH.

Between the structural genes for ketothiolase

and acetoacetyl CoA reductase of Chromatium
vinosum, two open reading frames (ORF4 and ORF5)
appear, and downstream from the reductase gene an
ORF7 has been identified (Figure 5). No additional
5 ORFs were identified in the PHA coding region of
A.eutrophus.

3. Expression of C.vinosum PHB synthetic genes in other bacteria.

With fragments PP10 and EP94 the ability to
10 synthesise PHB could be restored to PHB negative
mutants of A.eutrophus. Recombinant strains of the
PHB negative mutant A.eutrophus PHB-4, transformed
with these fragments, were able to synthesise
polymers containing 3-hydroxybutyrate and
15 3-hydroxyisovalerate at significant proportions,
when supplied with appropriate substrates.

Studies on expression of C.vinosum DNA
fragments in E.coli are presented in Table 1. Thus
E.coli transformed with plasmids containing
20 fragments PP10 and EP94 expressed PHB polymerase,
acetoacetyl CoA reductase and β ketothiolase
activities. They also synthesised PHB up to
between 10 and 12% dry weight. E.coli transformed
with plasmids containing fragment SE45 expressed
25 PHB polymerase and β ketothiolase, but not
acetoacetyl CoA reductase, and were unable to
synthesise PHB.

4. Polymer Biochemistry

The specific optical rotations of methyl
30 3-hydroxybutyric acid liberated by methanolysis of
PHB from C.vinosum (accumulated from acetate), from
A.eutrophus PHB-4 pHP1014::PP10 (accumulated from
fructose) and E.coli S17-1 pSUP202::PP10
(accumulated from glucose) were all negative. The

determined values of the specific optical rotation were similar to those for PHB isolated from A.eutrophus (accumulated from fructose).

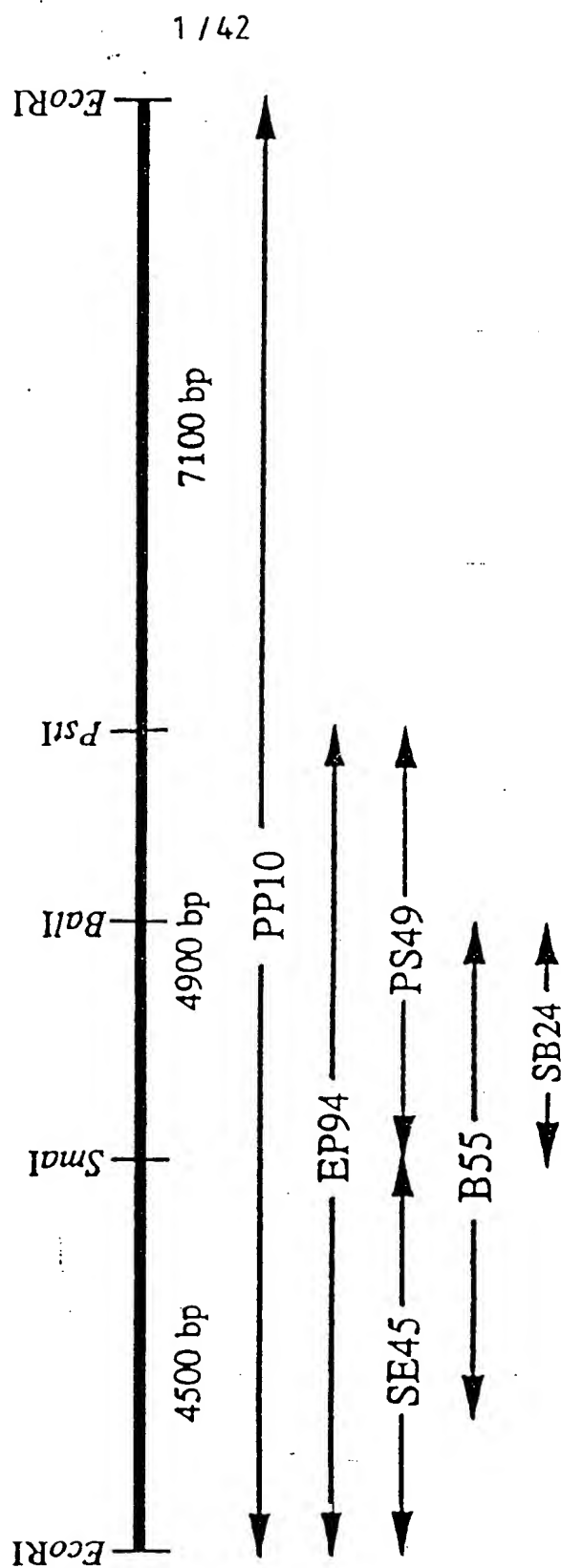
CLAIMS

1. Gene fragments isolated from the bacterium Chromatium vinosum and encoding polyhydroxy-alkanoate (PHA) synthase, acetoacetyl CoA reductase and β -ketothiolase.
2. Gene fragments as claimed in claim 1 in which the Chromatium vinosum is of the strain designated D, available to the public from the Deutsche Sammlung für Mikroorganismen under the Accession Number 180.
5
3. A 16.5kb EcoRI fragment of Chromatium vinosum DNA, designated PP10, hybridizable to a 5.2kb SmaI/EcoRI fragment, designated SE52 isolated from Alcaligenes eutrophus and known to contain the genes encoding PHA-synthase acetoacetyl CoA reductase and β -ketothiolase.
5
4. A fragment of the PP10 fragment claimed in claim 3, designated SE45, encoding the PHA-synthase and β -ketothiolase genes.
5. A fragment of the PP10 fragment claimed in claim 3, designated SB24, encoding the acetoacetyl CoA reductase gene.

6. A recombinant genome which contains one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.
7. A bacterium having incorporated in its genome one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.
8. A plant having stably incorporated in its genome by transformation one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.
9. A method for the manufacture of polyhydroxyalkanoates, comprising culturing the microorganism Chromatium vinosum, or a bacterium of a different species having stably incorporated within its genome by transformation one or more PHA synthesising genes from Chromatium vinosum.
10. A gene, encoding β -ketothiolase, having the nucleotide sequence shown in Figure 3.
11. A gene encoding polyhydroxyalkanoate synthase (phbC), having the nucleotide sequence shown in Figure 5.
12. A gene encoding acetoacetyl CoA reductase (phbB) having the nucleotide sequence shown in Figure 5.

FIG. 1

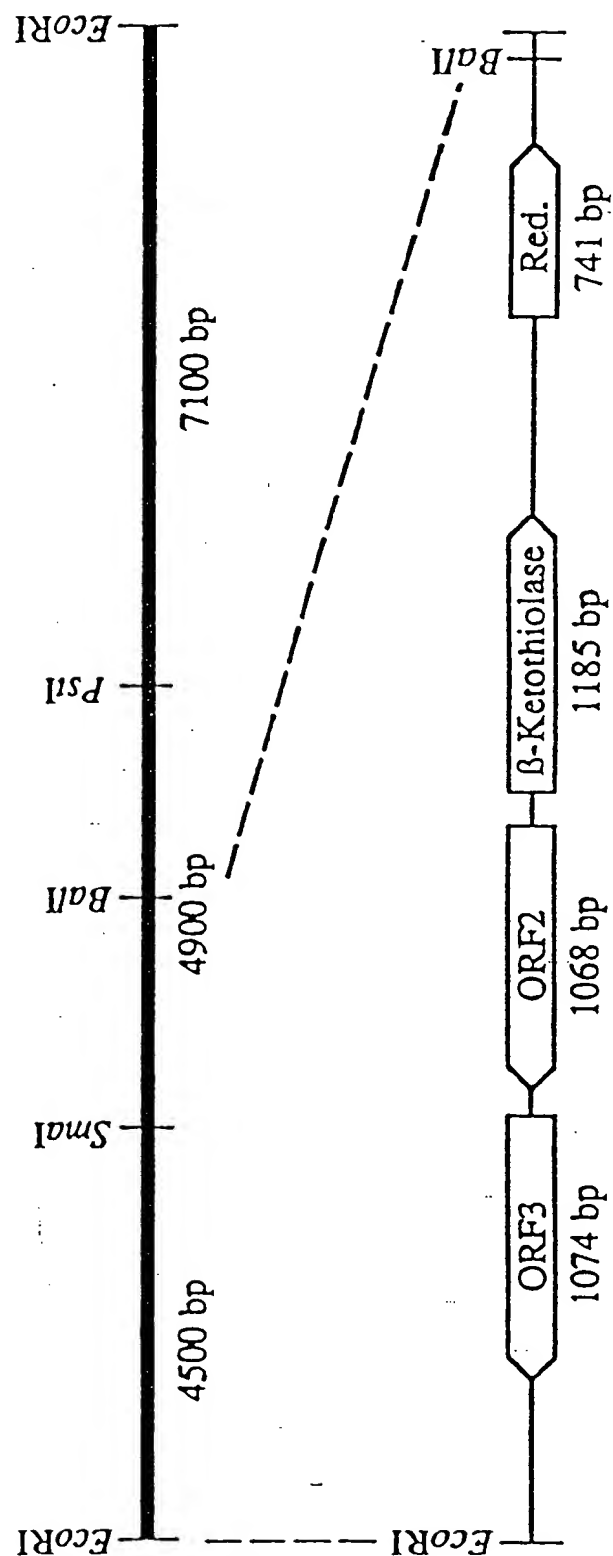
Physical map of the 16.5-kb *EcoRI* fragment PP10 and location of B55



2/42

FIG. 2

Region of β -ketothiolase and reductase locus and relevant open reading frames of PHB-synthase



3/42

FIG. 3 (1/9)

: Nucleotide sequence of fragment SE45

EcoRI

1 GCTTAAGTAGGTCCCGGTGATAGAGGTTGACGGCTCAAGCCTGAACTTG

101 CCGCGAAGTGTGCCCTGCCGACTAGGACTGAGGTAGGCTAGGCGTAGAG

201 TCACGAGGTAAGTAGGACCTCTTAGGTGTCGGCCCCCGCCCGCTGGCTC


301 GGCAGGCCTAAGCGCTACTGCAGTCTCGCCCCGCAAAGAGCGTATAAGC

401 GGGTGGGGCTACACAGCCACAGCGCGCGGGGACAGCCCGACCGCGCGC

501 ACGCCGCTCTGTGCAGGCGGGACCGAGACCCTGACCTTTGTCGTTTCCTC

601 ACGGCTGCGGCTACAAGAAGCTCGTCGAGTAAGCCCAGTCACCCTAGCC

701 AATTGCACTTCCGGTCGAGGTTCAAGCGAGCTACTCGCTGCTGCGGGCC

801  GCGGCCTTAACGAGGCGCCGAGCCCTTCGCAATAGCGAGCAACTCGGT
* R E N L W

901 CGGCTACACTGGCGGGCCCTTCCGGTCGAGCCACATCAGGCCCCGACGAC
G H I G G P F A L E T Y D P S S

1001 TCGCGCTTCTACAAGTTCGTGGCCCGTCCACTACAGGAAGTCCAGGTGAA
L A F I N L V P C T I D K L D V

4/42

FIG. 3 (2/9)

AAGCTAAAGGTCTGGGACAGTGTACTTTGGGCTATCAGACGAGTACTCATG

CGCGGCCTACAGCTAAACAAGTAGCTTCCTGACTCGGCCGGACCTAAGGAC

GGAAACGCTGACCGCGGCCGAAGGACTCAGATGACTGGAAACGGCCGACGC

GTAGGGCGGTCAGTCAGTCGTAGGTTCCAACTACCAACCGGCATTGTGCGC

GACCACCTTGTGACCCGGTGCGTAGGCGTACGGCCGCACGTTACAGGCCGG

CTAAGCCTGCGCGGAAGCACGTCTAGCTTCTCCAGAAAGGCCCACTCCGTC

GACCGCTGTAAAGATGTAGGTCCTAACTTAGTTTCGGACCATATCCCCGCGG

ACTCAGGGCTCGCAGTAGCACCTACGGCAAGCTGCCGACTGCTGTGCTCG

GAATGGCTACCGGCCGCACTGAAGAAAGACGCGGAACGGCGACTGTATCTA

K G I A P T V E K Q A K G S V Y I

CAGTCAGGGAAGTTCGCGGGATCTCCGTAGGCCGCCGTGGTCCACTAGGACC

T L G K L A R S A D P P V L H D Q

GGACAGGCGGGTCGTGGTGCGGCGGTAACCTTCGGCAACAAGACCATCT

E Q G G L V V G G N L F G N N Q Y

5/42
FIG. 3 (3/9)

1101 TCAGGAACTACTTGACTGCCTTCCAGAGCGGCCGGACTAGGCCCGACA
F D K I F Q R F T E G A Q D P S

1201 CTCGTCTAGTTGGTACAACCTGCATGAAGACTGGGCAGTCCGACTTTCC
L L D V M N V Y K Q G T L S F P

1301 CACAGCTGGCGGTCTAGCTACAGCTGCAAGACGTGGGTCCGGCTCTCG
T D V A L D I D V N Q V W A S L

1401 CGTGGAACAGGCCCCACGTCGCGCGACATGTAGTCCGACTTCCGCGGCG
R V K D P H L A S Y M L S F A G

1501 TATCAGGTGCGTCGCCAGCTACATCGGCAACTACATCAGCAGCTCGCA
Y D V C R D I Y G N I Y D D L T

1601 ACCGGCCACCGGTCGTCAGGGAACCTACCACCTCGCCAGAAGGACCTAC
Q G T A L L G K I T S R D E Q I

1701 CGACCCGAAGCCCGCGCGGGAGCCCAGCCAGTATCGCTATCTCCTGGT
P Q A E P A G E P R D Y R Y L V

1801 GAGCCGCAACTCGTCTAAGAGGTACGGGACTGGGTGGAACGCCGACAT
E A N L L N E M G Q G L K R S Y

S/D

1901 TAGCGACAGGAGACTAAGACAGACCACTCGGACGACAAGCTAGTCGGC

2001 GGAACCGCCGGCCCCGCCCCGCTGGACTCGCGTCACCGTCCGAACCACC
A K A A P A P S R L A T A P K T

6/42

FIG. 3 (4/9)

GCTTCTAGGTAAAGAGGTACGCGTCCTTCAAGAACTGGAACAGGCCCCAGCAG
P F I W K E M R L F N K V K D P D D

GAACTCGCTGTCCTTCCAGGTCAAGTCGTCGAGTGGCCCCTACAACGGGTAC
K L S L F T W N L L E G P I N G M

TCCAACAGGCCCCAGAACTTCAGCTGGCCGCACTGGTACCAGTGCTCCAACG
L N D P T K F D V P T V M T V L N

GGACTGTCTATGGCTCGTCTAACTGGAACAGCTGCGGCACCCGAAGCGCGTC
G Q C I G L L N V K D V G H A E R L

CTCGCGGCCAGCGGGACTAGGCCTATCGGGGTCAGCTAGTCCATCTGCAGG
L A R D A Q D P Y G W D I L Y V D

AGCCAGTACATCCCGGCCAACTGGTCCCGCATCTGCTAGTCCTCGCCGTGCC
D T M Y P R N V L A Y V I L L P V

CGAACAGGAGCGACATCTGCCGGACGAACCCCGACTGCGGCCACAGCTACCG
L K D E S Y V A Q K P S V G T D I A

CAGCTCGTAGAGGACCCACTGAAACAGGCCCGCCTACAGCTACCCCTTGTTAC
D L M E Q T L K D P R I D I P F M

GAACTGCGAGTTAGCCGGCCTAACCAGAACCACCACGCGGCCGCACGGCCTC
* D A P N T K T T R R R A P

GGCCGAGGAGTGGCCGGTCCCGACGCTGCGCTGCAAGGTCTAGCGCGTCCGA
A P E E G A L A A V R R E L D R L S

ORF3
←

7142

FIG. 3 (5/9)

2101 CACTGCGTCCCAGGAACAAGAGCGCTGCCAGAGGACCTCGGCCAGGACC
H R L A K N E R R T E Q L R D Q

2201 AGCTGGTCCTAGCTGTACGCGAAGAACTCGCGGTAGACTCGCAACTGGT
D V L I S M R K K L A M Q A N V

2301 GCGGAAGCGTCGTCCGCTGGGTCAACAGCATCTCGCGCGCGCGGCTCAG
A G E C C A V W N D Y L A R A S D

2401 CTGGCTGAAGTACGGCTCGAACCACATCTGCAACCACATAAGGACCTCG
V S K M G L K T Y V N T Y E Q L

2501 AGGAGCGCCACATCGGCTCTGGCCCGCGGCTCTCCCGAGCTAGTTCTG
E E R T Y G L G P A S L A R D L

2601 CGCTGTAGCTCCTGTAGTACGCGACGGTCAACAGCTCGCCGTCGAGGGT
P S M S S M M R Q W N D L P L E W

2701 CTTGCGAAGACGTACAGAAGCTCGCAGAACCAGGTCTCGAGGTTCGGC
F R K Q M D E L T K T W L E L G

2801 TTCTTCAAGAACGGGACGAGGTAGTAGAAAAGGTACTTGCTCGCACTGT
F F N K G Q E M M K E M F S R S

2901 GGGTCCCGCACAAACGGCTTGACCTCCGCGACAGGTCTGGGTACCGGAA
E W P T T A S S S A S D L G M A K

3001 TAGCAGTAACTTCTTTAATCACAACGAGTACCTGCATGGGAGGAGCACC
D D N F F N T N S M

← ORF2

S/D

8/42

FIG. 3 (6/9)

TCCCACGCGTCGAGCGACGCCCAGCCGTACAAGTTACGCGGGTCCAAGAGC
L T P L E S R T P M N L A G L N E

CGGCCGGCACCTACGCCCCGATAAGGCCACACCGCTGGAGCAGCCGTATCC
L R G H I R A Y E P T A V E D A Y

CTACCAAAACGGCGACAGCTACTGTGGGACCTACATCCTCGGGTACACGAG
I T K G S D I V G Q I Y S G M H E

CGCCGGACTATGAGGTACCGCGACGCGTAGTCGAGGACTATGACCGAGACG
A A Q Y E M A S R M L E Q Y Q S Q

ACAGGAACTTGACGAGCACCCCGTACAACGCGTCGTACAGCGGGCCGTAGC
S D K F Q E H P M N R L M D G P M

CTTGCTGTAGTCCGCGACGTACCACAACGGCGGCAGCAGCTCGCTCGGCGA
F S M L R Q M T N G G D D L S G S

AACGGCCGCAACGGGAGCAGCCGCGCAAACCGCTTCCACAGCCGCTCTGCC
N G A N G E D A R K A F T D A L R

CTAGGCCCCGCGCCCGCGGTACCGAAAGGTGGTGACTAGCTACCGGCGGA
L D P A A P A M A K W W Q D I A A

CGCCGAGTACAGCCAGGTCAACAGGGTCATGAACGCAACGTCAAGCTCGGT
R S M D T W N D W Y K R Q L E L W

← mRNA
GCGCACCACAACCCGCTTGCCATAGTACACCTAAACACGTGACGTTGTTTC

"-35" Ketothiolase

"-10"

ORF2

"-35"

9/42

FIG. 3 (7/9)

"-10"

3101 TACGCATCTTAGGTGTCCGGCGCTGGGGTATAGCCCCTGCGAGCAGGTA
V D A G R S A I G T F G G S L S
3201 GCAGCTGCGGCCGGCGTCACGGTAGCCTTGGAAGCCGCCGTCAGACAGC
R T G L A P E Q I D E V I L G Q V
3301 GCATGGCCTGAGCGCGGCCTTGTCTAGCTGCTCCACTAAGAGCCGGTCC
G L P H S V P A M T I N K V C G
3401 GCGCCGATGGCGTAAGCCACGGCCGGTACTGGTAGTTGTTCCAGACGCC
A D I V I A G G Q E S M S Q S S
3501 ACGGCTGTAGCAGTAGCGGCCGCCAGTCCTCTCGTACTCGGTCAGGAGC
K D T M I V D G L W D A F N N Y H
3601 TTCCTGTGGTACTAGCAGCTGCCGGAGACCCTACGGAAGTTGTTGATAG
Q Q D A F A A A S Q Q K T E A A
3701 TCGTCGTCCTGCGGAAGCGGCGGCGGAGCGTCGTCTTCTGGCTCCGGCG
R K G D P K V F D A D E F P R H
3801 CGCGTTCCCGCTAGGCTTCCACAACTACGGCTGCTCAAGGGCGCAGTG
G S V T A G N A S G I N D G A A M
3901 CCGTCGCAGTGCCGCCCATTTGCGGAGGCCGTAGTTGCTGCCCCGCCGGT
A R L V A F A S A G V D P A I M
4001 ACCGCGCAGACCACCGGAAGCGGTGCGGCCACAGCTAGGCCGCTAGTA

10/42

FIG. 3 (8/9)

S/D Ketothiolase
M S E N I V I
AAGGTTAGTCTTAGGTCTCCTGTAGGTGCGGTACTCGCTCTTGTAGCAGTA
S L S A T E I G T A V L K G L L A
AGTGAGAGCCGGTGGCTCTAGCCGTGGCGGCACGAGTTCCCCGACGACCGC
L T A G V G Q N P A R Q T T L H A
ACGACTGGCGGCCGCACCCGGTCTTGGGGCGGGCAGTCTGGTGCACGTGC
S G L K A V H L A M Q A I A C G D
GTCGCCAGACTTCCGCCACGTAGACCGCTACGTCCGGTAGCGGACGCCCT
H V L P R S R D G Q R M G D W S M
GTGCAGGACGGCGCAAGCGCGCTGCCAGTCGCGTACCCGCTGACCAGCTAC
M G T T A E N I A Q K Y G F T R E
TGTACCCGTGGTGGCGGCTCTTGTAGCGGGTCTTCATGCCGAAATGCGCGC
Q K A G R F Q D E I I P I E I P Q
CGTCTTCCGACCGGCGAAGGTCCTGCTCTAGTAAGGCTAGCTCTAAGGCGT
G T T A E S L G K L R P A F S K D
CCGTGGTGCCGGCTCTCAGACCCGTTGACGCAGGCCGGAAGAGCTTCCTG
V V V M K E S K A K E L G L K P M
ACCAGCACCCTACTTCCTCAGGTTCCGGTTCCTTGACCCAGACTTCGGCT
G T G P I P A S T K C L E K A G W
CCCCTGCCCCGGGCTAGGGCCGCAGCTGGTTCACGGACCTCTTCCGGCCGAC

11/42
FIG. 3 (9/9)

4101 T P A D L D L I E A N E A F A A
CTGGGGCCGCCTAGACCTAGACTAGCTCCGGTTGCTTCGGAAGCGGCGC

4201 V N G G A I A I G H P I G A S G A
CAGTTGCCGCCGCGGTAGCGGTAGCCAGTAGGCTAGCCGCGGAGGCCAC

4301 G L A T L C I G G G Q G V A L A
TCCCAGACCGCTGCGACACGTAGCCGCCGCCGGTCCCGCACCGCGACCG

4401 TCGGAGGACTTAGCGAGGTCCGTGACTTGCGGGACGGCTAGGGCCTAGC

SmaI
4501 GGGCCC

FIG. 3

Q A M S V N Q D M G W D L S K V N
GTCCGGTACAGCCAGTTGGTCCTGTACCCGACCCTAGACAGGTTCCAGTTG

R V L V T L L Y E M Q K R D A K K
GCGCGCACGAGCACTGGGACGAGATACTCTACGTCTTCGCGCTGCGGTTCT

V E R M *
CCAGCTCGCCTACACTCGGCAGCAGGCGGCCAGACTTAGCGGCCGCCTGGC

CACCCCGCAAACGCGCGAACCCCATCTGAACGGCTTGCTGGTCGGCTTGGC

12/42

FIG. 4 (1/3)

PHB polymerase	173	E	S	G	G	E	S	L	R	A	G	V	R	N	M	E	D	L	T	R	-	-	G	K	I	S	Q	T	D	E	S	A	F	E	V	-							
ORF 3	1	M	F	P	I	D	I	R	P	D	K	L	T	Q	E	M	L	D	Y	S	R	-	-	K	L	G	Q	G	M	E	N	L	N	A	E	-							
PHA polymerase	149	E	T	G	G	K	S	L	L	D	G	L	S	N	L	A	K	D	L	V	N	N	G	G	M	P	S	Q	V	N	M	D	A	F	E	V	-						
PHA polymerase	249	N	S	G	G	Q	S	L	V	F	G	V	A	H	L	L	D	D	L	R	H	N	D	G	L	P	R	Q	V	D	E	R	A	F	E	V	-						
PHB polymerase	206	G	R	N	V	A	V	T	E	G	A	V	V	F	E	N	E	Y	F	Q	L	Y	R	Y	K	P	L	T	D	-	-	K	V	H	A	R	P	P	P	P			
ORF 3	34	A	I	D	T	G	V	S	P	K	Q	A	V	Y	S	E	D	K	L	V	L	I	Q	Y	D	R	P	E	G	A	P	E	A	Q	P	P	P	P	P				
PHA polymerase	184	G	K	N	L	G	T	S	E	G	A	V	V	Y	R	N	E	L	E	L	I	Q	Y	K	P	I	T	E	-	-	Q	V	H	A	R	P	P	P	P				
PHA polymerase	284	G	G	N	L	A	A	T	A	G	A	V	V	F	R	N	E	L	E	L	I	Q	Y	K	P	M	S	E	-	-	K	Q	H	A	R	P	P	P	P				
PHB polymerase	240	L	L	M	V	P	P	C	I	N	K	Y	Y	I	L	D	L	Q	P	E	S	S	L	V	R	H	V	V	E	Q	Q	T	F	F	L	L	V	V					
ORF 3	70	L	L	L	V	Y	A	L	V	N	R	P	Y	M	T	D	I	Q	E	E	R	S	S	T	I	K	G	L	L	A	T	G	Y	D	V	L	L	I	I				
PHA polymerase	1218	L	L	L	V	P	P	Q	I	N	N	K	F	Y	F	D	L	S	P	E	K	S	L	A	R	Y	C	L	R	S	Q	Q	T	F	F	L	L	I	I				
PHA polymerase	2218	L	L	L	V	P	P	Q	I	N	N	K	F	Y	F	D	L	S	P	E	K	S	L	A	R	Y	C	L	R	S	Q	Q	T	F	F	L	L	I	I				
PHB polymerase	276	S	W	R	N	P	P	D	A	S	M	A	G	S	T	W	D	D	Y	I	E	H	A	A	I	R	A	I	E	V	A	R	D	I	S	G	Q	Q	D	D	D	D	
ORF3	106	D	W	G	Y	P	P	D	Q	A	D	R	A	L	T	L	D	D	Y	I	N	-	A	A	I	R	C	V	D	Y	L	R	E	A	H	G	V	V	V	V	V	V	
PHA polymerase	1254	S	W	R	N	P	P	T	K	A	Q	R	E	W	G	L	S	T	Y	I	D	-	A	L	K	E	A	V	D	A	V	L	A	I	S	G	G	V	V	V	V	V	V
PHA polymerase	2254	S	W	R	N	P	P	D	P	R	H	R	E	W	G	L	S	S	Y	V	Q	-	A	L	E	E	A	L	N	A	C	R	S	I	S	G	G	V	V	V	V	V	V

FIG. 4(2/3)

PHB polymerase 312 K I N V L G F C V G G T I V S T A L A V L A A R G E - H P A A S V T L L
 ORF3 142 K V N L L G I C Q Q S G G A F - - S L M Y S A L H P D - K V R N L V T M V
 PHA polymerase 1288 D L N M L G A C C A G G L T C T A L V G H Y A A L G E - N K V N A L T L L
 PHA polymerase 2288 D P N L M G A C C A G G L T M A A L Q G H L Q A K H Q L R R V R S A T Y L

PHB polymerase 347 T T L L L D F F - A D T G I L D V F V D E G H V Q L R E A T L G G A G A P -
 ORF3 174 T P - V D F F K T P D N L S A W V Q N V D I D L A V D I M G G A G E -
 PHA polymerase 1323 V S V L D T - T M D N Q V A L F V D E Q T L E A - - - A K R R S Y Q
 PHA polymerase 2324 V S L L D S - K F E S P A S L F A D E Q T L E A - - - A K R R S Y Q

PHB polymerase 382 C A L L R G L E L A N T F S F L R P N D L V W N Y V V D N Y L K G N T P
 ORF3 208 - L L N W T F L S L K P F S L T G Q K Y V N M V D L L D D P D K V K N F
 PHA polymerase 1353 A G V L E G S E M A K K V F A W M R P N D L I W N Y W V N N Y L L G N E P
 PHA polymerase 2354 R G V L D G A E V A R I E A W M R P N D L I W N Y W V N N Y L L G K T P

PHB polymerase 418 V P F D L L F W N G D A T N L P P G P W Y C W Y L R H T Y L Q N N E L K V P
 ORF3 243 L R M E K - - W I F D S P D Q A G E T F R Q F I K D F I Q N N G F - L N
 PHA polymerase 1389 P V F D I L F W N N D T T R L P A A F H G - D L I E M F K S N P L T R P
 PHA polymerase 2390 P A F D I L Y W N N A D S T R L P A A L H G - D L L D F F K L N P L T H P

FIG. 4 (3/3)

14/42

PHB polymerase	454	G	K	L	T	V	C	G	V	P	P	V	D	L	A	S	I	D	V	P	T	Y	I	Y	G	S	R	E	D	H	I	V	P	W	T	A	A	Y				
ORF3	276	G	G	V	V	L	G	G	Q	E	E	V	D	L	K	D	I	T	C	P	V	L	N	I	F	A	L	Q	D	H	L	V	P	P	D	A	S	R				
PHA polymerase	1424	D	A	L	E	V	C	G	T	P	I	D	L	K	Q	V	K	C	D	I	Y	S	L	A	G	T	N	D	H	I	T	P	W	Q	S	C	Y					
PHA polymerase	2425	A	C	L	E	V	C	G	T	P	I	D	L	Q	K	V	E	L	D	S	F	T	V	A	G	S	N	D	H	I	T	P	W	D	A	V	Y					
PHB polymerase	490	A	S	T	A	L	L	A	N	-	-	-	-	-	-	-	-	-	K	L	R	F	V	L	C	A	S	G	I	A	G	V	I	N	P	P	A	K	N	K		
ORF3	312	A	L	K	G	L	T	S	S	P	D	Y	T	E	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
PHA polymerase	1460	R	S	A	H	L	F	G	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
PHA polymerase	2461	R	S	A	L	L	L	G	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
PHB polymertase	521	R	S	H	W	T	N	D	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
ORF3	348	I	G	K	W	L	N	E	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
PHA polymerase	1491	A	R	F	M	T	G	A	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
PHA polymerase	1492	A	Y	Y	L	A	N	P	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

15 / 42

FIG.5 (1/20)

EcoRI

1 GCTTAAGTAGGTCCTCCGGTGATAGAGGTTGACGGCTCAAGCCTGAACCTTGAA

101 CCGCGAAGTGTCCTCCCTGCCGACTAGGACTAGGCTAGGCTAGGCGTAGAGCG

201 TCACGAGGTAACTAGGACCTCTTAGGTGTCGGCCCCCGCTGGCTCGG

301 GGCAGGCCTAAGCGCTACTGCAGTCTCGCCCCCGCAAGAGCGTATAAGCGT

401 GGGTGGGGCTACACAGCCCCACAGCGCGGGGACAGCCCGACCGCGCGGA

501 ACGCCGCTCTGTGCAGGGGGACCGAGACCCCTTGACCTTTGTGTTCCCTCCT

601 ACGGCTGCGGCTACAAGAAGCTCGTCGAGTAAGCCCCAGTCACCCCTAGCCGA

701 AATTGCACTTCCGGTCGAGGTTTCAGGCGAGCTACTCGCTGCTGGGGGCCAC

801 GCGGCCCTTAACGAGGCGCGCGAGCCCTTCGCAATAGCGAGCAACTCGGTGA

* R E N L W

16/42

FIG. 5 (2/20)

GCTAAAGGTCTGGGACAGTGTACTTTGGGCTATCAGACGAGTACTCATG
CGGCTACAGCTAAACAACCTAGCTTCCTGACTCGGCGGACCTAAGGAC
AAACGCTGACCGCGCGGCGAAGGACTCAGATGACTGGAACGGCCGACGC
AGGCGGTCAGTCAGTCGTAGGTTCCAACTACCAACCGGCATTGTGCGC
CCACCTTGTAACCGGTGCGTAGGCGTACGGCCGACGTTACAGGCCGG
AAGCCTGCGCGGAAGCACGTCTAGCTTCTCCAGAAAGGCCCACTCCGTC
CCGCTGTAAAGATGTAGGTCCTAACTTAGTTCTGGACCATATCCCCCGCG
TCAGGGCTCGCAGTAGCACCTACGGCAAGCTGCCGACTGCTGTCGCTCG
ATGGCTACCGCGCCTGAAAGAAAGACGCGGAACGGCGACTGTATCTA
K G I A P T V E K Q A K G S V Y I

17/42

FIG. 5 (3/20)

901	CGGCTACACTGGCGGCCCTTCCGGTCGAGCCACATCAGGCCCGACG G H I G G P F A L E T Y D P S
1001	TCGCGCTTCTACAACCTCGTGGCCCGTCCACTACAGGAAGTCCAGGTG L A F I N L V P C T I D K L D V
1101	TCAGGAAC TACTGACTGCCTTCCAGAGCGCGGACTAGGCCCGAC F D K I F Q R F T E G A Q D P S
1201	CTCGTCTAGTTGGTACAAC TGCATGAAGACTGGGCAGTCCGACTTTC L L D V M N V Y K Q G T L S F
1301	CACAGCTGGCGGTCTAGCTACAGCTGCAAGACGTGGTCCGGCTCTC T D V A L D I D V N Q V W A S L
1401	CGTGGAACAGGCCCGACGTGCGCGGACATGTAGTCCGACTTCCGCGGC R V K D P H L A S Y M L S F A G
1501	TATCAGGTGCGTCCCGCAGCTACATCGGCAACTACATCAGCAGCTCGC Y D V C R D I Y G N I Y D D L

18/42

FIG. 5 (4/20)

ACCAGTCAGGGAAC TCGCGGATCTCCGTAGGCCCGCGTCCACTAGGACC
S T L G K L A R S A D P P V L H D Q

AAGGACAGCGGGTCGTGGTGGCGGTAAC TCTTCGGCAACAAGACCATCT
E Q G G L V V G G N L F G N N Q Y

AGCTTCTAGGTAAGAGGTACGCGTCCTTCAAGAACTGGAACAGGCCCAGCAG
P F I W K E M R L F N K V K D P D D

CGAACTCGCTGTCCCTCCAGGTCAAGTCGTGAGTGGCCCTACAACGGGTAC
P K L S L F T W N L L E G P I N G M

GTCCAACAGGCCCCAGAACTTCAGCTGGCCGCACTGGTACCAGTGCTCCAACG
L N D P T K F D V P T V M T V L N

GGGACTGTCTATGGCTCGTCTAACTGGAACAGCTGCGGCACCCGAAGCGGTC
G Q C I G L L N V K D V G H A E R L

ACTCGCGGCCAGCGGACTAGGCCCTATCGGGGTACGTAGTCCATCTGCAGG
T L A R D A Q D P Y G W D I L Y V D

19/42

FIG. 5 (5/20)

1601 ACCGGCCACCGGTCGTCAGGGAACCTACACCTCGCCAGAGGACCTA
Q G T A L L G K I T S R D E Q I

1701 CGACCCGAAGCCGCGGGAGCCAGCCAGTATCGCTATCTCCTGG
P Q A E P A G E P R D Y R Y L V

1801 GAGCCGCAACTCGTCTAAGAGGTACGGACTGGTCGAACGCCGACA
E A N L L N E M G Q G L K R S

1901 TAGCGACAGGAGACTAAGACAGACCACCTCGGACGACAAAGCTAGTCGG
s/d

2001 GGAACCGCGCGCCCGCGCTGGACTCGCGTCACCGTCCGAACAC
A K A A P A P S R L A T A P K T

2101 CACTGCGTCCGGAACAAGAGCGCTGCCAGAGGACCTCGGCCAGGA
H R L A K N E R R T E Q L R D

2201 AGCTGGTCCTAGCTGTACGCGAAGAACTCGCGGTAGACTCGCAACTG
D V L I S M R K K L A M Q A N V

20/42

FIG. 5^(6/20)

CAGCCAGTACATCCCGGCCAACTGGTCCCGCATCTGCTAGTCTCGCGTGCC
D T M Y P R N V L A Y V I L L P V

T M Y P R N V L A Y V I L L P V

TCGAACAGGAGCGACATCTCGCGGACGAACCCGACTGGGCCACAGCTACCG
L K D E S Y V A Q K P S V G T D I A

L K D E S Y V A Q K P S Y G T D I A

TCAGCTCGTAGAGACCCACTCAAAACAGGGCGGCTACAGCTACCCCTTGTA
Y D L M E Q T L K D P R I D I P F M $\xrightarrow{\text{phbC}}$

Y D L M E O T L X D P R I D I P E M

→ phbc

CGAACTGCGAGTTAGCCGGCCTAACCAAGAACCAACGCGCGACGGCCTC
* D A P N T K T T R R R A P

*.D.A.P.N.T.X.T.T.R.R.R.A.P

CGGGCGAGGAGTGGCCGGTCCCGACGCTGGCTGCAAGTCTAGCGGTCGGA
A P E E G A L A A V R R E L D R L S

APPEAL AVAILABLE

Q L T P L E S R T P M N L A G L N E
CCTCCACGGTCGAGCGACGCCAGCCGTACAAGTACGGGGTCCAAGAGC

Q L T P L E S R T P M N L A G L N E

L R G H I R A Y E P T A V E D A Y
GTGGCGGGCACCTACGCCCGCATAAGGCCACACCGCTGGAGCAGCGTATCC

L R G H I R A Y E P T A V E D A Y

21/42

FIG. 5 (7/20)

2301 GCGGAAGCGTCGTCGCTGGGTCAACAGCATCTCGCGCGCGGCTC
A G E C C A V W N D Y L A R A S

2401 CTGGCTGAAGTAGGGCTCGAACACACATCTGCAACCACATAAGGACCT
V S K M G L K T Y V N T Y E Q

2501 AGGAGCGCCACATCGGCTCTGGCCCCGGGCTCTCCCGAGCTAGTTC
E E R T Y G L G P A S L A R D L

2601 CGCTGTAGCTCCTGTAGTAGCGGACGGTCAACAGCTCGCCGTCGAGG
P S M S S M M R Q W N D L P L E

2701 CTTGCGAAGACGTACAGAAGCTCGCAGAACAGGTCTCGAGGTTCC
F R K Q M D E L T K T W L E L

22/42

FIG.5 (8/20)

AGCTACCAAAACGGCGACAGCTACTGTGGGACCTACATCCTCGGGTACACGAG
D I T K G S D I V G Q I Y S G M H E

CGCGCCGACTATGAGGTACCGGACGCGTAGTCGAGGACTATGACCGAGACG
L A A Q Y E M A S R M L E Q Y Q S Q

TGACAGGAACTTGACGAGCACCCCGTACAAACGCGTCGTACAGCGGCCGTAGC
S D K F Q E H P M N R L M D G P M

GTCTTGCTGTAGTCCGCGACGTACCACAACGGCGGACGAGCTCGCTCGGCGA
W F S M L R Q M T N G G D D L S G S

GCAACGGCCGCAACGGGAGCAGCCCGGCAACCGCTTCCACAGCCGCTCTGCC
G N G A N G E D A R K A F T D A L R

FIG. 5^(9/20)

23/42

2801 TTCTTCAAGAACGGGACGAGGTAGTAGAAAGGTACTTGCTCGCAGTGT
 F F N K G Q E M M K E M F S R S

2901 GGGTCCCGCACAAACGGGCTTGACCTCCGGGACAGGTCTGGGTACCGGAA
 E W P T T A S S S A S D L G M A K

3001 ATCGTCATTGAGAAATTAGTGTGCTCATGGACGTACCCCTCCTCGTGG
 TAGCAGTAACTTCTTTAATCACAAACGAGTACCTGCATGCGAGGAGCACC
 D D N F F N T N S M S/D

← ORF2

"-10" mRNA

3101 ATGCGTAGAATCCACAGGCCGCGACCCCATATCGGGGACGCTCGTCCAT
 TACGCTTCTTAGGTGTCCGGCGCTGGGTATAGCCCTCGGAGCAGGTA

3201 V D A G R S A I G T F G G S L S
 CGTCGACGCCGCGCAGTGCCCATCGGAACCTTCGGCGGCAGTCTGTCTG

3301 R T G L A P E Q I D E V I L G Q V
 CGTACCGGACTCGCGCGGAACAGATCGACGAGGTGATTCTCGGCCAGG

3401 G L P H S V P A M T I N K V C G
 CGGGGCTACCGCATTCGGTGCCGCCCATGACCATCAACAAGGTCTGCGG

FIG. 5^(10/20)

24/42

CTAGGCCCCCGCCGGTACCGAAGGTGGTGA CTAGTACCGGCGGA 5'
 L D P A A P A M A K W W Q D I A A

CGCCGAGTACAGCCAGGTCAACAGGGTCATGAACGCAACGTCAAGCTCGGT 5'
 R S M D T W N D W Y K R Q L E L W

CGCGTGGTGTGGGCGAACGGTATCATGTGGATTGTGCACTGCAACAAAG 3'
 GCGCACCAACAACCCGCTTGCCATAGTACACGCTAAACACGTGACGTGTTTC 5'
 mRNA " -10" " -35"

phbA →
 S/D M S E N I V I
 TTCCAATCAGAATCCAGAGGACATCCAGCCCATGAGCGAGAACATCGTCAT 3'
 AAGGTTAGTCTTAGGTCTCCTGTAGTGCGGTACTCGCTCTTGTAGCAGTA 5'

S L S A T E I G T A V L K G L L A
 TCACTCTGGCCACCGAGATCGGCACCGCCGTGCTCAAGGGGCTGCTGGCG 3'

L T A G V G Q N P A R Q T T L H A
 TGCTGACCGCGCGGTGGGCCAGAACCCCGCCGTCAGACCCAGCTGCACG 3'

S G L K A V H L A M Q A I A C G D
 CAGCGGTCTGAAGCGGTGCATCTGGGATGCAGGCCATCGCCTGCGGGGA

25 / 42

FIG. 5 (11/20)

3501	A D I V I A G G Q E S M S Q S S H
	TGCCGACATCGTCATCGCGGGGTCAGGAGAGCATGAGCCAGTCTCTCGC
3601	K D T M I V D G L W D A F N N Y H
	AAGCACCATGATCGTCGACGGCCTCTGGGATGCCTTCAACAACATCA
3701	Q Q D A F A A S Q Q K T E A A
	AGCAGCAGGACGCCCTTCGCCCGCCCTCGCAGCAGAACCGAGGCCGCG
3801	R K G D P K V F D A D E F P R H G
	GCGCAAGGCGCATCCGAAGGTGTTGATGCCGACGAGTTCCCGCGTCACG
3901	G S V T A G N A S G I N D G A A M
	GGCAGCGTCACGGGGGTAACGCCCTCCGGCATCAACGACGGGGCGGCCAT
4001	A R L V A F A S A G V D P A I M
	TGGCGCGTCTGGTGGCCTTCGCCACGCCGGTGTCGATCCGGCGATCATG
4101	T P A D L D L I E A N E A F A A Q
	GACCCCGCGGATCTGGATCTGATCGAGGCCAACGAAGCCTTCGCCGCGC

26/42

FIG.5 (12/20)

V L P R S R D G Q R M G D W S M
ACGTCTGCCGCGTTCCGCGACGGTCAGCGCATGGGCGACTGGTCCGATG

M G T T A E N I A Q K Y G F T R E
CATGGCACCAACCGCGAGAACATCGCCAGAAAGTACGGCTTACGGCGG

Q K A G R F Q D E I I P I E I P Q
CAGAAGGTGGCGCTTCCAGGACGAGATCATTCGGATCGAGATTCCGCA

T T A E S L G K L R P A F S K D
GCACCAGGCCGAGAGTCTGGGCAAGCTGCGTCCGGCCTTCTCGAAGGAC

V V V M K E S K A K E L G L K P M
GGTCGTGGTGAAGGAGTCCCAAGGCCAAGGAAGTGGGTCTGAAGCCGA

G T G P I P A S T K C L E K A G W
GGGACGGGCCCGATCCCGCGGTCCGACCAAGTGCCTGGAGAAGCCGGCTG

A M S V N Q D M G W D L S K V N
AGGCCATGTCGGTCAACCAGGACATGGGCTGGGATCTGTCCAAGGTCAAC

27/42

FIG. 5 (13/20)

4201 V N G G A I A I G H P I G A S G
GTCAACGGCGGCCCATCGCCATCGGTCATCCGATCGGCGCTCCGG

4301 G L A T L C I G G Q G V A L
AGGTCTGGGACCGCTGTGCATCGGCGGCCAGGCGGTGGCGTG

4401 AGCCTCCTGAATCGCTCCAGGCACTGAACGCCCTGCCGATCCCGGAT
→

ORF4 →

4501 SmaI M N S E R I I K K Y P N
CCCGGTGCCCATGAACAGCGAGCGGCATCATCAAGAAGTATCCGAAC

4601 D L V M S G Q P F R V L D S A N
CGATCTGGTGATGAGCGGACAGCCCTTCCGCGTCTCGACAGGCCA

4701 E T G G Q P L F S A N M L A Q I
ACCGCGGTGAGCGCGTGTTCAGCGCCAACATGCTGGCCAGATCAT

28/42

FIG. 5 (14/20)

A R V L V T L L Y E M Q K R D A K K
 TGGCGCGTGCTCGTGACCCCTGCTCTATGAGATGCAGAAGCGGACGCCAAGA

A V E R M *
 GCGGTCGAGCGGATGTGAGCCGTCGTCGCCGGTCTGAATCGCCGGGACCG

CGGTGGGGCGGTTGGCGCGCTTGGGGTAGACTTGCCGGAACGACCGCAACCG

R R L Y D T E V S R Y I T L A D V R
 CGCGCCTCTACGACACCGAGGTCAGCCGCTATATCACCCCTCGCCGATGTGCG

D S D I T R S I L L Q I M L E E E
 ATGACAGGGATATCACCCGTTCCATCCTGCTCCAGATCATGCTGGAGGAGGAG

I R F Y G G T L Q G T F A R Y L E S
 CCGCTTCTACGGGGCACCCCTTCAGGGCACCTTCGCCCGCTATCTGGAATCTT

29/42

FIG. 5 (15/20)

4801 S L D L F A K Q Q Q E V T K A
CACTCGACCTGTTCCGCAAGCAGCAACAGGAAGTGACCAAGGCACTC

4901 I W A D L Q D E L M R A A G F P
CTGGGCTGATCTCCAGGACGAACACTCATGCGCGGCTTGCTTCCGG

5001 GCGTCGGTCACAGCTTTATTGTGCAATGCAACATTGCTGCACTGCA
"-10" (?)

5101 E W T N K S V E R M T S F G E
ACGAGTGGACCAACAAGAGCGTCGAGCGCATGACCAGCTTCGGTGAG

5201 L Y M D H S M R L M K L A T E S
CCTGTACATGGATCACAGCATGCGCCTGATGAAGCTGGCCACCGAGT

5301 S E R V M A E S K A T M Q F F G
AGCGAGCGGTCATGGCCGAGAGCAAGGCCACCATGCAGTTCTTCGG

5401 E D L R K S V A V *
GCGAAGATCTGCGCAAGAGCGTCGCCGCTCTAAAGACGCCGACCTCTG

FIG. 5 (16/20)

L T D N P F G T V T R L T Q K N V E
 ACCGACAATCCCTTCGGGACGGTGACACGCCCTGACTCAGAAGAACGTCGAGAT

V A P R K K E * " -35" (?)
 TCGCGCCGCGCAAGAAAAAAGAAATAATGAGGATTGCGAAATTTGCGCTTGACG

ORF5
 S/D M N T T D S L K T V N
 CAAACCTTACGGAGAGATGATCATGAACACCCACGACAGCCTCAAGACCGTCA

L N V R L F E K L A A R Q M D A V N
 CTGAACGTGCGTCTGTTCGAGAAAGCTGGCCGCCGTCAGATGGACGCCGTGAA

K G Y N D L F K G Q V D A T K E L
 CCAAGGGTTACAATGACCTCTTCAAGGGTCAGGTCGACGCCCAAGAACTG

D A R D E Y R V W F E K S L N D V S
 CGATGCCCGGACGAATACCGCGTGTGGTTCGAGAAGAGCCTGAACGACGTCA

GGCCATCGCGATCCAGGGATGGATCGCCATTGGTCATGCTTCCGGATCGGCCG

30/42

31/42

FIG. 5 (17/20)

5501 GGAGCAGCGCCCAATGGAACCAACGCTTCACCTTGCCCTGCCGCTTGGA
5601 AAGATTCCCTGAGGGAACCCCATGGCTCGTATCGCACTCGTCACCGGCG
5701 T V V A N C H P S E A A A E E
5801 D V S S F D D S A R M V R E I T
5901 K T F K K M E Q A H W E A V I N
6001 L E R G F G R I I N I S S V N G
6101 H G F T M A L A Q E G A S K G V

32/42

FIG. 5 (18/20)

GTAAAGTGGCCTTGAAGTTCGACGACACTGTTTCATCGTCTCTCAATAGTTCCA

I G G I G T S I C T R L A K D G C
GCATCGGGGCATCGGCACTTCGATCTGCACACGCCCTGGCAAAGGATGGCTG

W K Q A R A A E G F D I A V F T A
GTGGAAGCAGGCCCGTGGCCCGGAGGGTTTCGACATCGCCGCTTTCACCGCT

E Q V G P I D I L V N C A G I T R D
GAGCAGGTGGTCCCATCGACATCCTGGTCAACTGTGCCGGCATCACCCGCG

V N L N S V F N V T R Q V W D G M
ACGTCAACCTCAACAGCGTCTTCAACGTCACCCGTCAGGTGTGGGACGGGAT

Q R G Q F G Q A N Y S A A K A G M
TCAGCGGGCCAGTTCGGTCAGGCCAACTATTCCGCCGCCAAGGCCGTATG

T V N T I S P G Y V E T A M T L A M
ACCGTCAACACCATCTCGCCCGGCTATGTCGAGACGGCCATGACCCCTGGCGA

FIG. 5^(19/20)

33 / 42

6201 N D D V R N S I I S G I P M R
TGAACGACGATGTGGCAACAGCATCATCAGCGGTATTCCGATGCG

6301 E S G Y M T G A N L P V N G G
CGAGAGCGGTATATGACGGGGCCCAATCTGCCGGTCAACGGCGGT

6401 GCCCGGTGTTTCAGGATCTCACCAGTCTCCTCGTCTCTCATC
ORF7 →
M N A V M T D V R D L I

6501 CGCCCATCCGATGAACGCTGTGATGACCGAGTACGCGATCTGATC

6601 G L Q V E G E R P L Q R L V S G
GGTTGCAAGTGGAGGGCAACGGCCGCTCCAGCGGTGGTGGG

6701 I L V H H G W F W K N E N P C
CCATTCTGGTCCATCATGGCTGGTTCTTGGAGAAGCAATCCCTG

6801 L I A Y H L P L D A H P E L G
TCTGATCGCCTATCATCTGCCGCTCGATGCCATCCCGAATCGGC

6901 L A N G L L W A A I G S A H D A
CTGGCCAATGGTCTGCTGTGGGGCGGATTGGCTCAGCCCATGACG

34/42

FIG. 5 (20/20)

R M A Q P N E I A A A I A F L A G D
TCGCATGGCTCAGCCTAATGAGATCGCCGCCCATCGCTTTCCTGGCCGCGA

L F M H *

CTGTTTCATGCATTGATTAGATCATACCGGGCCGAATACAAACACTGACAATG

ATGAGACGTTTACAGCCCGCGCCAGCCGGGCTTTTTTTGTGTAGAAATCGAAT

R Y C D D V L D A A R F A D Y A P N
CGCTACTGCGATGACGTGCTCGACGCGCGCGCTTCGCCGACTATGCCGCCGAAT

V T A S A A L I E A A I A E H A D A
GCGTGACGGCCAGCGCGCGTTGATCGAGGCCGCGATCGCGGAGCAGCCGACG

L I G I K G Q R A R T L L S A G V S
CCTGATCGGCATCAAGGGGCGAGCGCGCAAGGACATTGCTCAGCGGGGTGTGAG

N N A T L G R R L D F I D M E P T A
AACAATGCCACACTCGGTCCGCGCTCGATTTCATCGACATGGAACCGACCGCA

C V L H G A C L A
CCTGCGTCCCTTCAGGAGCATGTCTCGCATC

FIG. 6
(1/4)

35/42

C.v.	1	M S E N	I V I V D A	G R S	A I G	T F G G S L S S	L S A T E	I G T A
A.e.	1	M T D V	V V I S A	A R T	A V G	F F G G S L A K	P E L L A V	G G A V
Z.r.	1	M S T P S	I V I A S	A A R	A V G	F F G G S L A K	P E L L A V	G G A T
E.c.	1	M E Q V	V I V D A	I R T	A V G	F F G G S L A K	P E L L A V	G G A H
S.u.	1	M S Q N	V Y I V S	A R T	A V G	F F G G S L S S	P E L L A V	G G A A
R.n.	1	M A L L R G V F	I V A A K	A R T	A V G	F F G G S L S S	P E L L A V	G G A F
C.v.	34	V L K G L L A R	- T G L	- A P E Q I D E V I L G Q V	- A P E Q I D E V I L G Q V	- A P E Q I D E V I L G Q V	- A P E Q I D E V I L G Q V	- A P E Q I D E V I L G Q V
A.e.	33	V I K A A L E R	- A G V	- K P E Q V S E V I L G Q V	- K P E Q V S E V I L G Q V	- K P E Q V S E V I L G Q V	- K P E Q V S E V I L G Q V	- K P E Q V S E V I L G Q V
Z.r.	34	V I S A V L E R	- A G V	- A A G E V N E V I L G Q V	- A A G E V N E V I L G Q V	- A A G E V N E V I L G Q V	- A A G E V N E V I L G Q V	- A A G E V N E V I L G Q V
E.c.	34	L M R S L L A R	- N P A L	- E A A A L D I Y W G C V	- E A A A L D I Y W G C V	- E A A A L D I Y W G C V	- E A A A L D I Y W G C V	- E A A A L D I Y W G C V
S.u.	34	A E K G A L A K	- V P E L	- A S K D F D E I I F G N V	- A S K D F D E I I F G N V	- A S K D F D E I I F G N V	- A S K D F D E I I F G N V	- A S K D F D E I I F G N V
R.n.	35	A A R A A L S A	- G K V	- P P E T I D S V I V G N V	- P P E T I D S V I V G N V	- P P E T I D S V I V G N V	- P P E T I D S V I V G N V	- P P E T I D S V I V G N V
C.v.	66	- P A R Q T T L H	A G L P H S V P A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M
A.e.	65	- P A R Q A A I K	A G L P A M V P A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M
Z.r.	66	- P A R Q A A M K	A G L P A M V P A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M
E.c.	68	- I A R Q N A A L	A G L P A M V P A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M
S.u.	68	- P A R Q V A L L	A G L P A M V P A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M
R.n.	68	Y L A R H V G L	A G L P A M V P A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M	T I N K V C G S G L K A V H L A M
C.v.	101	Q A I A G G D A D T V	I A G G Q E S M S S Q S	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G
A.e.	100	N A I M A G D A E I V	I A G G Q E S M S S Q S	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G
Z.r.	101	Q Q I M A G D A S I V	I A G G Q E S M S S Q S	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G
E.c.	103	R M I M T G D A Q A C L V V	I A G G Q E S M S S Q S	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G
S.u.	103	Q S I K C G N A D V V	I A G G Q E S M S S Q S	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G
R.n.	104	Q E I C S K D A E V V	I A G G Q E S M S S Q S	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G	S H V L P R S S R D G Q R M G

36/42

FIG.6 (2/4)

C.v.	137	-	D	W	S	M	K	D	T	M	I	V	D	G	L	W	D	A	F	N	N	Y	H	M	G	T	T	A	E	N	I	A	K	K	Q	W	Q	I	Y	G	F
A.e.	136	-	D	A	K	L	V	D	T	M	I	V	D	G	L	W	D	V	Y	N	Q	Y	H	M	G	T	T	A	E	N	V	A	K	K	Q	W	Q	I	Y	G	F
Z.r.	136	-	D	F	K	M	I	D	T	M	I	K	D	G	L	T	D	A	F	Y	G	Y	H	M	G	T	T	A	E	N	V	A	K	K	Q	W	Q	I	Y	G	F
E.c.	135	-	G	L	S	-	R	N	V	A	K	A	A	G	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
S.u.	139	-	Q	T	V	L	I	D	G	V	E	R	D	G	L	N	D	A	Y	D	G	L	A	M	G	V	H	A	E	K	A	R	D	W	D	I	I	I	I	I	
R.n.	140	L	D	L	K	L	E	D	T	L	W	-	A	G	L	D	T	Q	H	V	K	L	P	M	G	M	T	A	E	N	L	A	Q	Y	N	I	I	I	I		

C.v.	172	T	R	E	Q	Q	D	A	F	A	A	S	Q	Q	K	T	E	A	A	Q	K	A	G	R	F	Q	D	E	I	I	P	I	E	I	P	Q
A.e.	171	T	R	E	A	Q	D	A	F	A	V	G	S	Q	N	K	A	E	A	Q	K	A	G	K	F	D	E	E	I	V	P	I	P	I	P	Q
Z.r.	171	S	R	D	E	Q	D	A	F	A	V	A	S	Q	N	K	A	E	A	Q	K	D	G	R	F	K	D	E	E	I	V	P	I	P	Q	
E.c.	161	S	P	E	M	Q	D	A	F	A	R	S	H	A	R	A	W	A	A	T	Q	S	A	A	F	F	K	N	E	I	V	P	I	P	Q	
S.u.	174	T	R	D	Q	Q	D	S	E	A	I	E	S	Y	Q	K	S	Q	Q	S	Q	K	E	G	F	D	N	E	I	V	P	I	P	Q		
R.n.	175	S	R	E	D	C	D	R	Y	A	L	Q	S	Q	R	W	K	A	A	N	E	A	G	Y	F	N	E	E	M	A	P	I	E	V	K	T

C.v.	208	R	K	G	D	P	R	V	F	D	A	D	E	F	P	R	H	G	T	T	A	E	S	L	G	K	L	R	P	A	F	-	S	K	D	-	G	
A.e.	207	R	K	G	D	P	V	A	F	K	T	D	E	F	V	R	Q	G	A	I	L	D	S	M	S	G	L	K	P	A	F	-	S	K	D	-	G	
Z.r.	207	R	K	G	D	I	T	V	-	D	A	D	E	F	Y	I	R	H	G	A	L	D	S	M	S	G	L	K	P	A	F	-	S	K	D	-	G	
E.c.	197	A	D	G	V	L	K	Q	F	N	Y	D	E	F	V	I	R	P	E	T	T	V	E	A	L	A	T	L	R	P	A	F	-	S	K	D	-	G
S.u.	210	F	R	G	K	P	D	T	Q	V	T	N	D	E	E	P	A	R	L	H	V	E	A	L	A	K	S	A	R	T	V	F	-	S	K	D	-	G
R.n.	211	K	K	G	K	Q	T	M	-	Q	V	D	E	H	A	R	P	Q	T	T	L	E	Q	L	Q	N	L	P	P	V	F	-	S	K	D	-	G	

37/42

FIG.6 (3/4)

C.v.	242	V	T	A	G	N	A	S	G	I	N	D	G	A	A	M	V	V	M	K	E	S	K	A	K	E	L	G	L	K	P	M	A	R	L
A.e.	241	T	V	T	A	G	N	A	S	G	L	N	D	G	A	A	A	V	V	M	S	A	A	K	A	K	E	L	G	L	T	P	L	A	T
Z.r.	240	T	V	T	A	G	N	A	S	G	L	N	D	G	A	A	A	V	V	M	S	E	A	E	A	S	R	R	G	I	Q	P	L	G	R
E.c.	232	M	T	T	A	G	T	S	A	L	S	D	G	A	A	A	A	A	L	M	S	E	S	R	A	H	E	L	G	I	K	P	R	A	I
S.u.	245	T	V	T	A	G	T	S	A	L	S	D	G	A	A	A	A	A	M	L	V	S	E	R	V	L	K	E	K	N	L	P	L	A	V
R.n.	244	T	V	T	A	G	N	A	S	G	M	S	D	G	A	G	V	V	I	I	A	S	E	D	A	V	K	K	H	N	F	T	L	A	R

C.v.	278	V	A	F	A	N	T	V	V	A	H	L	P	A	D	F	T	W	A	P	S	L	A	V	I	T	G	A	L	L	K	K	A	G	L	
A.e.	277	K	S	Y	A	T	V	V	G	C	D	P	A	I	M	G	T	G	P	T	P	A	S	T	K	C	R	A	L	L	K	K	A	G	L	
Z.r.	276	V	S	W	A	V	V	G	C	D	P	P	K	V	M	G	T	G	P	T	P	A	S	T	K	C	R	A	L	L	K	K	A	G	L	
E.c.	268	R	S	M	A	V	V	G	C	D	P	P	K	V	M	G	T	G	P	T	P	A	S	T	K	C	R	A	L	L	K	K	A	G	L	
S.u.	281	K	G	W	G	E	A	H	L	P	A	D	F	T	W	A	P	S	L	A	V	I	T	G	A	L	L	K	K	A	G	L	S	L	K	
R.n.	280	V	G	Y	F	V	S	G	C	D	P	A	I	M	G	T	G	P	T	P	A	S	T	K	C	R	A	L	L	K	K	A	G	L	S	L

C.v.	313	D	L	I	E	A	N	E	A	F	A	A	Q	A	M	S	V	N	Q	D	M	G	W	D	L	-	-	-	-	-	-	-	-	-	-
A.e.	312	D	L	I	E	A	N	E	A	F	A	A	Q	A	L	A	V	N	Q	D	M	G	W	D	T	-	-	-	-	-	-	-	-	-	
Z.r.	311	D	L	I	E	A	N	E	A	F	A	A	Q	A	C	A	V	N	Q	D	M	G	W	D	P	-	-	-	-	-	-	-	-	-	
E.c.	303	G	V	F	E	M	N	E	A	F	A	A	Q	I	L	P	C	I	K	D	L	G	L	I	E	Q	I	D	-	-	-	-	-	-	
S.u.	317	D	Y	F	E	F	N	E	A	F	S	V	G	L	V	N	T	K	I	L	K	L	D	P	-	-	-	-	-	-	-	-	-	-	
R.n.	315	D	L	I	E	A	N	E	A	F	A	A	Q	F	L	A	V	Q	K	S	L	D	L	D	P	-	-	-	-	-	-	-	-	-	

FIG.6 (4/4)

38/42

C.v.	346	I	A	I	G	H	P	I	G	A	S	G	A	R	V	L	V	T	L	L	Y	E	M	Q	K	R	D	A	K	K	G	L	A	T	L	C	I
A.e.	345	I	A	I	G	H	P	I	G	A	S	G	A	R	V	L	V	T	L	L	H	E	M	K	R	R	D	A	K	K	G	L	A	T	L	C	I
Z.r.	344	I	A	I	G	H	P	I	G	A	S	G	A	R	V	L	V	T	L	L	F	E	M	K	R	R	D	A	K	K	G	L	A	T	L	C	I
E.c.	339	I	A	L	G	H	P	I	G	C	S	G	A	R	V	L	V	T	L	L	N	L	M	E	R	K	D	V	Q	F	G	L	A	D	G	V	
S.u.	350	V	A	L	G	H	P	I	G	C	S	G	A	R	V	V	T	L	L	S	I	L	Q	E	G	G	K	I	G	V	A	A	I	C	N		
R.n.	348	I	A	L	G	H	P	I	G	G	S	G	S	R	I	T	A	H	L	V	H	E	L	R	R	R	G	G	K	Y	A	V	G	S	A	C	I

Homology to β -ketothiolase of *C. vinosum*:

C.v.	382	G	G	-	G	Q	E	V	A	L	A	V	E	R	M	68.2%
A.e.	381	G	G	-	G	M	G	V	A	L	A	V	E	R	K	59.4%
Z.r.	380	G	G	-	G	M	G	V	A	M	C	I	E	S	L	43.0%
E.c.	375	S	G	L	G	Q	G	I	A	T	V	F	E	R	V	42.7%
S.u.	387	G	G	-	G	G	A	S	S	V	V	I	E	K	L	42.6%
R.n.	384	G	G	-	G	Q	G	I	S	L	I	I	Q	N	T	A

Comparison of amino acid sequences of the β -ketothiolases encoded by *Chromatium vinosum* (C.v.), *Alcaligenes eutrophus* (A.e.), *Zoogloea ramigera* (Z.r.), *Escherichia coli* (E.c.), *Saccharomyces uvarum* (S.u.) and *Rattus norvegicus* (R.n.).

39/42

FIG. 7 (1/2)

C.v.	1	M	A	R	I	A	L	V	T	G	G	I	G	T	S	I	C	T	R	L	A	K	D	G	C	T	V	V	A	N	G	H	P							
A.e.	1	M	T	Q	R	I	A	Y	V	T	G	G	I	G	T	A	I	C	Q	R	L	A	K	D	G	F	T	V	V	A	N	G	C	G						
Z.r.	1	M	S	R	V	A	L	V	T	G	G	S	R	G	I	G	A	I	S	I	A	L	K	A	A	G	Y	K	V	A	A	S	Y	A	G					
C.v.	36	S	E	A	A	A	E	E	W	K	Q	A	R	A	A	E	G	F	D	I	A	V	F	T	A	-	-	-	-	-	-	-	-	-	D	V	S	S	F	
A.e.	37	-	N	S	P	R	R	E	K	W	L	E	Q	Q	K	A	L	G	F	D	F	I	A	S	E	G	-	-	-	-	-	-	-	-	-	N	V	A	D	W
Z.r.	36	-	N	D	-	-	-	-	-	-	-	D	A	A	K	P	-	-	-	-	F	K	A	E	T	G	I	A	V	-	Y	K	W	D	V	S	S	Y		
C.v.	66	D	S	A	R	M	V	R	E	I	T	E	Q	V	G	P	I	D	I	L	V	N	C	A	G	I	T	R	R	K	T	V	M	F	K	K	M	T		
A.e.	66	D	S	T	K	T	A	F	D	K	V	E	A	D	L	G	E	P	I	D	V	L	I	N	N	A	G	I	T	R	D	V	M	F	K	K	M	T		
Z.r.	60	E	A	C	V	E	G	I	A	K	V	E	A	D	L	G	E	P	I	D	V	L	V	N	N	A	G	I	T	K	D	A	M	F	K	K	M	T		
C.v.	102	Q	A	H	E	A	V	I	N	V	N	I	N	S	V	L	F	N	V	T	R	Q	Q	Y	I	N	C	M	L	E	R	G	E	F	G	R	I	I	V	V
A.e.	102	R	A	D	W	D	A	V	I	N	T	N	I	T	S	L	F	N	V	T	K	Q	V	I	N	N	A	G	I	T	R	D	V	M	F	K	K	M	T	
Z.r.	96	P	D	Q	R	N	A	V	I	N	T	N	I	T	G	L	F	N	M	T	H	P	V	N	S	G	M	R	D	R	S	F	F	G	R	I	I	V	V	

FIG. 7 (2/2)

40/42

C.v.	138	I S S V N G Q Q R G Q F G Q Q A N Y S A A K A G M H G F T M A L A Q E G A S	
A.e.	138	I S S V N G Q Q K G Q F G Q Q T N Y S T A K A G L H G F T M A L A Q E V A T	
Z.r.	132	I S S I N G Q Q K G Q M G Q A N Y S A A K A G D L G F T K A L A Q E G A A	
C.v.	174	K G V T V N T I S P G Y V E T A M T L A M N D D V R - N S I I S G I P M	
A.e.	174	K G V T V N T I S P G Y I A T D M V K A I R Q Q D V L - D K I I V A T I P V	
Z.r.	168	K G I T V N A I C P G Y I G T E M V R A I P E K V L N E R I I P Q I P V	
C.v.	209	R R M A Q Q P N E I A A A I A E L A G D E S G Y M T G A N L P V N G G L F E	
A.e.	209	R R L G L P E E I A S I C A W L S E E S G F S T G A D F S L N G G L H	
Z.r.	204	G L R G E P E E I A R I V V F L A S D E A S F I T G S T I S A N G G Q E	
C.v.	245	M H	Homology to reductase of <i>C. vinosum</i> :
A.e.	245	M G	56.4%
Z.r.	240	F V	48.3%

Comparison of amino acid sequences of reductases encoded by
C. vinosum (C.v.), *A. eutrophus* (A.e.) and *Z. ramigera* (Z.r.).

41/42

FIG. 8 (1/2)

Strain (plasmid)	relevant markers	Medium
S17-1 (pHP1014)	none	LB-Tc-Glu
S17-1 (pHP1014::PP10)	<i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁺ , ORF2 ⁺	LB-Tc-Glu
S17-1 (pHP1014::EP94)	<i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁺ , ORF2 ⁺	LB-Tc-Glu
S17-1 (pSUP202)	none	LB-Tc-Glu
S17-1 (pSUP202::PP10)	<i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁺ , ORF2 ⁺	LB-Tc-Glu
XL1-Blue (KS ⁻)	none	LB-Ap-Glu
XL1-Blue (KS ⁻)	none	LB-Ap-IPTG
XL1-Blue (KS ⁻ ::SE45 ⁺)	<i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺	LB-Ap-Glu
XL1-Blue (KS ⁻ ::SE45 ⁺)	<i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺	LB-Ap-IPTG
XL1-Blue (KS ⁻ ::SE45 ⁻)	<i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺	LB-Ap-Glu
XL1-Blue (KS ⁻ ::SE45 ⁻)	<i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺	LB-Ap-IPTG
XL1-Blue (KS ⁻ ::B55)	<i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁻ , ORF2 ⁺	LB-Ap-Glu
XL1-Blue (KS ⁻ ::B55)	<i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁻ , ORF2 ⁺	LB-Ap-IPTG

42/42

FIG. 8 (2/2)

Specific activity (U/g of protein)				Accumulation of
PHB synthase	β -Ketothiolase	Acetoacetyl-CoA reductase		PHB
		NADH-dependent	NADPH-dependent	(% of cellular dry weight)
<0.1	<20	<20	<20	<0.1
6.1	1190	420	60	10.2
5.2	940	310	60	9.7
<0.1	<20	<20	<20	<0.1
6.1	1320	320	60	12.1
<0.1	<20	<20	<20	<0.1
<0.1	<20	<20	<20	<0.1
6.0	1120	<20	<20	<0.1
4.3	980	<20	<20	<0.1
4.8	470	<20	<20	<0.1
2.9	70	<20	<20	<0.1
<0.1	80	30	<20	<0.1
<0.1	1870	610	40	<0.1

INTERNATIONAL SEARCH REPORT

International Application No

PCT/G8 92/01291

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12N15/52; A01H5/00;	C12N15/53; C12P7/62	C12N15/54; C12N1/21
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12N ; C12P ; A01H	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	ARCHIVES OF MICROBIOLOGY vol. 155, no. 5, 1991, pages 415 - 421 LIEBERGESELL, M., ET AL. 'Formation of poly-3 hydroxyalkanoates by phototrophic and chemolithic bacteria' see the whole document ---	1-12
A	TRENDS IN BIOTECHNOLOGY vol. 5, no. 9, September 1987, pages 246 - 250 BYROM, D. 'Polymer synthesis by microorganisms: technology and economics' see page 248, left column; table 2 ---	1-12
A	WO,A,9 100 917 (MIT) 24 January 1991 see the whole document ---	1-12
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IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
09 OCTOBER 1992	19. 10. 92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	MADDOX A.D.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,8 900 202 (MIT) 12 January 1989 see the whole document ---	1-12
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 264, no. 26, 15 September 1989, BALTIMORE, MD US pages 15298 - 15303 PEOPLES, O.P., ET AL. 'Poly-beta-hydroxybutyrate (PHB) biosynthesis in Alcaligenes eutrophus H16' see figure 4 ---	1-12
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ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

GB 9201291
SA 62148

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